The present invention relates to polypeptides, muteins, etc., which comprise His968. Such polypeptide fragments can be useful to prepare antibodies, to inhibit lipid kinase activity by competing for substrates, etc. Useful polypeptides include, e.g., polypeptides which consist of about 500, 200, 100, 50, 30, 20, 10, 8, etc., the activation loop (e.g., amino acids 964-988, 950-988), etc. These polypeptides can be effective in eliciting an immune response to amino acid His968, and flanking regions thereof.

A PI3Kγ polypeptide mutein, comprising a sequence having at least 95% amino acid sequence identity to Fig. 3, and having a His968. Such a mutein can lack any of the mentioned activities of PI3K, but still retain its lipid kinase activity.

The present invention also relates to a method of modulating Ras activity in activating the PI3Kγ. Ras binding to PI3Kγ through its Ras binding domain ("RBD") leads to enzyme activation. The regions involved in the Ras interaction with PI3Kγ have been identified to include, e.g., a) specific regions of the N-terminal lobe of the catalytic region, such as kβ1-kβ2, kβ4-kβ5; kα6; b) RBD regions, such as Rα2 and Rβ3 -Rβ4; and c) RBD residues, such as Lys234, Asp238, and Lys255. Modification of any these regions or specific residues can be effective in modulating Ras activity, e.g., inhibiting, blocking, decreasing, reducing, enhancing, increasing, etc., its activity. By the phrase, "Ras modulatory activity," it is meant any activity in which PI3K affects Ras, including binding to Ras, activating Ras, etc. Ras modulatory activity can be measured conventionally, e.g., as described in Bondeva et al., Science, 282:293-296, 1998.

The present invention also relates to PI3K polypeptides and muteins thereof which relate to the Ras modulatory activity. For instance, polypeptides involved in Ras modulatory and/or binding activity to PI3K, includes, e.g., kβ1-kβ2 (782-794), kβ4-kβ5 (816-825); kα6 (921-942); RBD regions, such as Rα2 (291-300) and Rβ3 - Rβ4 (276-278, and fragments which comprise residues, such as Lys234, Asp238, and Lys255, e.g., Useful polypeptides include, e.g., polypeptides comprising, consisting of, consisting essentially, 782-816, 782-906, 795-816, 809-906, 817-906, and various combinations of any of the mentioned regions. A PI3K mutein can comprise 100% amino acid sequence identity with Fig. 3 at one or more of the above-mentioned domains, and less than 100% identity, such as 85%, 90%, 95%, 99%, or more, at any

other regions of the PI3K. Since such mutein comprises the sequences that are used to interact with Ras, the mutein would possess Ras modulatory activity, but can lack other activities. A preferred Ras binding domain polypeptide consists essentially amino acid residues 220-311, having Lys234, Asp238, and Lys255, but less than 100% amino acid sequence identity at other positions, e.g., 70%, 75%, 80%, 85%, 90%, 95%, 99%, or more, at any other regions of the PI3K.

Antibodies, or other specific ligands, can be used to block Ras binding to PI3K, or the activation that results from such interaction. Regions a) and b) are involved in the intramolecular interaction of the N-terminal catalytic lobe with the RBD. Antibodies to any of such regions can block Ras interaction. Residues of RBD, such as Lys234, Asp238, and Lys255, form bonds with Ras (See, Table). Useful antibodies which block the Ras binding to PI3K, preferably include antibodies which are specific for a peptide comprising amino acids Lys234, Asp238, and, Lys255. In addition, antibodies to any of the above-mentioned regions which are involved with Ras modulatory can also be used.

The present invention also relates to methods of inhibiting the binding of PI3Kγ to cell membranes, comprising, e.g., modifying an amino acid a) the lining the crevice region between the N- and C-lobes (about residues 844-950, especially residues 844, 847, 947, 948, and 950 which form part of the phospholipid head-group pocket); b) the CBR regions (about residues 371-380, 401-407 and 434-459); or c) the region comprising the activation loop (about residues 964-989, especially 967). As already mentioned, "modifying" can mean replacing or chemically modifying amino acids in the mentioned domains, or contacting with ligands, such as antibodies, which recognize specifically the mentioned domains.

Additional surface residues of PI3K are, e.g., about 755-756, 807-808, 994-905. and 1077-1084. Thus, inhibiting or modifying any of these residues, as discussed above and below, are useful to prevent binding of PI3K to cell membranes.

Polypeptide fragments or muteins of PI3K which possess cell membrane binding activity can be used as modulators of the cell-membrane binding activity. For instance polypeptide fragments coding for regions a), b), and/or c), or parts thereof, can be administered in vivo or in vitro as antagonists to prevent an endogenous enzyme from targeting to cell membranes. Antibodies specific-for these regions can

be used in the same way. A preferred polypeptide mutein has about 100% sequence identity with regions a), b), and/or c), and less than 100%, e.g., 99%, 95%, 90%, 85%, 80%, 70%, or more, sequence identity with the remaining regions of a PI3K shown in Fig. 3.

PI3K has a helical domain consisting of five A/B pairs of anti-parallel helices. Much of the B-surface is solvent exposed, providing a surface for interaction with other proteins, such as the p101 adaptor or Gβγ subunits. The present invention thus relates to modulating protein-protein interactions with PI3Kγ, comprising modifying the surfaces of the B-helices. The B-surfaces comprise, the exposed parts of B-helices, hB1 (570-577), hB1' (579-586), hB2 (601-613), hB2' (615-619), hB3 (638-650) hB4 (676-686), or hB5 (710-722). The modifying can comprise contacting said amino acid with an antibody specific-for hB1, hB1', hB2, hB2', hB3, hB4, or hB5, or by replacing, substituting, deleting, modifying, etc., an amino acid at such regions. A preferred polypeptide mutein or fragment (e.g., consisting essentially of 570-722) of a PI3Kγ comprises 100% sequence identity to hB1-hB5 of Fig. 3, and less than 100% sequence identity to the remaining sequence in Fig. 3, e.g., 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 99%, etc. Such mutein would retain the ability to interact with proteins.

Nucleic acids which code for any of the polypeptides, polypeptide fragments, and muteins thereof, can be prepared conventionally, using naturally-occurring or synthetic nucleotide sequences. See, e.g., Maniatis et al., Molecular Cloning, A Laboratory Mammal, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1989.

Antibodies, e.g., polyclonal, monoclonal, recombinant, chimeric, humanized, single-chain, Fab, etc., can be prepared according to any desired method. See, e.g., screening recombinant immunoglobulin libraries (e.g., Orlandi et al., Proc. Natl. Acad. Sci., 86:3833-3837, 1989; Huse et al., Science, 256:1275-1281, 1989); in vitro stimulation of lymphocyte populations (Winter and Milstein, Nature, 349: 293-299, 1991). For example, for the production of monoclonal antibodies, a polypeptide according to the present invention can be administered to mice, goats, or rabbits subcutaneously and/or intraperitoneally, with or without adjuvant, in an amount